QUANTITATIVE CHARACTERIZATION OF POLYCHLORINATED BIPHENYL MIXTURES (AROCLORS® 1248, 1254 AND 1260) BY GAS CHROMATOGRAPHY USING CAPILLARY COLUMNS

PHILLIP W. ALBRO*, JEAN T. CORBETT and JOANNA L. SCHROEDER

Laboratory of Environmental Chemistry, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709 (U.S.A.)

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SUMMARY

The polychlorinated biphenyl (PCB) compositions of Aroclors 1248, 1254 and 1260 have been determined using gas chromatography. A highly efficient glass capillary coated with the moderately polar Dexsil 410 served as the primary column. Pairs of isomers not resolved on Dexsil 410 were easily distinguished on short, less efficient capillaries coated with Silar 5C, Apiezon L, or OV-25. The advantages associated with the high selectivity of moderately polar columns outweighed the high column efficiencies associated with non-polar phases, permitting not-very-efficient columns to provide the needed information. Meaningful quantitation was obtained through the use of the hydrogen flame ionization detector. These Aroclors, now characterized in terms of the relative molar percentages of approximately 100 different PCBs, can be used as secondary standards to obtain relative molar responses for electron capture detectors. This will permit valid application of quantitative gas–liquid chromatography to environmental samples.

INTRODUCTION

Commercial mixtures of chlorinated biphenyls, previously manufactured in the U.S.A. under the trade name of Aroclors®, have been used extensively as dielectric fluids in transformers, as plasticizers, heat-exchange fluids, in microscope immersion oils, and in many other applications where their viscosities, heat resistance, insulating ability, or high refractive indices are advantageous[1,2]. Since the Aroclors and similar products manufactured in other countries consist of complex mixtures of isomers and may individually contain close to 100 components, most procedures in common use for the analysis of polychlorinated biphenyls (PCBs) attempt to measure the whole class without providing complete separation of individual components. Metabolic and environmental alteration of the PCBs found as pollutants and tissue residues result in a need to analyze mixtures for which there is no matching reference standard. Since routine quantitation using halogen-selective detectors or mass spectral ion currents is strongly influenced by differences in composition between sample and

calibration standard, the validity of such procedures has been questioned[3,4]. It is generally agreed that PCB quantitation would be valid if each component could be individually measured and summed to obtain a measure of "total PCB content". Such an approach has a significant advantage over "chemical summation", *e.g.* perchlorination[4] in that information can be obtained concerning levels of the more highly toxic or biologically active isomers that may be present.

Fractionation of PCB mixtures by gas chromatography on capillary columns has been evaluated as a means of providing complete analytical data[5,6]. Thus far, no one capillary column has provided sufficient resolution to distinguish all of the possible PCB isomers. Low-polarity liquid phases have traditionally been used (SE-30, OV-1, OV-101, SF-96, Dexsil 300 or Apiezon L), so the confirmatory changes in elution order that might be provided by polar phases have not been investigated. Schulte and Acker[7] obtained 200,000 theoretical plates in glass capillaries coated with SE-30, but these columns only resolved the Clophen® series of PCBs into 55 peaks. This may reflect the non-selectivity of the methyl silicones. Sissons and Welti[8] distinguished 77 components of Aroclors using an Apiezon L support-coated open tubular (SCOT) column, but many of the peaks could permit multiple assignments. Nuclear magnetic resonance and mass spectrometry were needed to confirm identifications.

Jensen and Sundström[9] attempted to provide complete quantitative analyses of Clophen A50, Clophen A60 and the PCBs extracted from human adipose tissue. They used 5.2-m packed columns of Apiezon L (purified) and SF-96 methyl silicone. Additional resolution was provided by prefractionation of the PCBs according to number of *o*-chlorines on charcoal. Unfortunately, quantitation was provided by an electron capture detector (ECD), whose response could only be calibrated for those isomers available in the form of standards. Since the ECD response to different PCB isomers is so highly variable[3], this approach involves considerable uncertainty.

Zell *et al.*[6] analyzed the Clophen series of PCBs on glass capillaries coated with OV-101. They provided quantitative data obtained using a hydrogen flame detector, which has been shown[10] to be rather constant in its response to different PCB isomers. These investigators found that several pairs of PCBs coeluted on OV-101, even at a resolution permitting retention indices to be given to five significant figures.

The retention behavior of PCBs is sufficiently different on different liquid phases that one can generally find a phase capable of resolving any given PCB pair[11]. By analyzing PCB mixtures sequentially on a series of packed columns of varying selectivities, we have been able to provide complete quantitative characterizations of Aroclors 1221, 1242, and 1016[11,12]. Six liquid phases were needed for Aroclor 1221, and twelve for the other two mixtures. The complexity of the higher Aroclors, 1248, 1254 and 1260, however, has frustrated our efforts to characterize them adequately through the use of packed columns.

In the present communication, we describe the compositions of Aroclors 1248, 1254 and 1260 as determined by quantitative gas–liquid chromatography using glass (or in one case, nickel) capillary columns of a range of selectivities. An approach similar to this could readily be used to analyze the altered PCB mixtures from animal tissues or environmental samples.

MATERIALS AND METHODS

Aroclor 1248, lot No. KA504, Aroclor 1254, lot No. KA38, and Aroclor 1260, lot No. KA1009 were gifts from Monsanto (St. Louis, MO, U.S.A.). The injection solvent was benzene ((Burdick & Jackson Labs., Muskegon, MI, U.S.A.) when an ECD was to be used, or methylene chloride (Burdick & Jackson Labs.) for the hydrogen flame ionization detector (HFID). The gas chromatograph was a Varian Model 3740 with pulsed ^{63}Ni ECD and HFID capabilities and equipped with the Varian split/splitless capillary inlet system. The carrier gas was helium throughout. Helium make-up gas was supplied to give 30 ml/min through the HFID, while 5% methane in argon was supplied as make-up for the ECD. For the latter, make-up gas was fed at 20 ml/min through the capillary outlet tee and at 10–20 ml/min through the ECD base. The detectors were maintained at 280°C and the sample inlet at 250°C. Samples of 1 μl (0.1 % to 1 % Aroclor, w/w) were injected either splitless for the HFID or with a 20:1 to 60:1 split for the ECD. The Varian ^{63}Ni ECD was operated in a constant current, variable frequency mode for maximum linear dynamic range.

The capillary columns used in this study were:

(A) 50 m × 0.25 mm I.D. glass, Dexsil 410 liquid phase, operated at 200°C with a helium flow-rate of 0.67 ml/min at 20 p.s.i.g. The split ratio was 45:1 for 1% Aroclor, with the ECD. This column, prepared commercially by Quadrex Corp. (New Haven, CT, U.S.A.), gave 175,000 effective theoretical plates for 2,3,5,2′,3′,5′-hexachlorobiphenyl. (Column efficiency always appears less for PCBs than for hydro-carbons because of the partial displacement of ^{37}Cl-containing from ^{35}Cl-containing isomers in the case of the PCBs.)

(B) 25 m × 0.25 mm I.D. glass, Silar 5C liquid phase, operated at 200°C with a helium flow-rate of 1.0 ml/min at 11 p.s.i.g. A 30:1 split ratio was used with the ECD. This column, also prepared commercially by Quadrex, gave approximately 51,000 effective theoretical plates for 2,3,5,2′,3′,5′-hexachlorobiphenyl.

(C) 24 m × 0.38 mm I.D. glass SCOT, Apiezon L on Tullanox 500, at 205°C with a helium flow-rate of 3.5 ml/min at 10 p.s.i.g. This column gave 28,000 effective theoretical plates for 2,3,5,2′,3′,5′-hexachlorobiphenyl and was made in-house.

(D) 15 m × 0.5 mm I.D. Nickel 200 SCOT OV-25 on Tullanox 500, 200°C isothermal with a helium flow-rate of 1.3 ml/min at 14 p.s.i.g. This column, also prepared in-house, gave only 15,000 effective theoretical plates for 2,3,5,2′,3′,5′-hexachlorobiphenyl.

Peak areas were measured using an Autolab System IV electronic analogue integrator. The HFID peak areas were corrected for relative molar response as described previously[10]. All results are averages of three runs. Retention indices were calculated with a retention time resolution of 1 sec (ref. 13).

Column A, Dexsil 410, was used as the master column. Pairs of isomers whose retention indices[11] would not permit their distinction on Dexsil 410 were, in almost every case, widely separated on Silar 5C, Apiezon L, or OV-25 .With only one or two exceptions, columns A and B used sequentially (separate runs) would permit the qualitative identification of all of the PCB isomers occurring to a significant extent in the Aroclors. Since we did not have all 209 possible PCB isomers available as reference standards, identification was based on co-chromatography (coincidence of retention indices) with the 45 standards available, and on "best fit" matching of summed half

indices as justified previously[11]. The Apiezon L and OV-25 columns then allowed us to detect any discrepancies there might be between assignments made on the basis of columns A and B, and the retention indices at which corresponding-sized peaks ought to elute on columns C and D. This corroboration was especially needed in the case of PCBs having more than three chlorine atoms in one or both rings, since these PCBs have multiple half-indices to choose between[8].

Further confirmation of the identifications was obtained by separating the Aroclors into fractions containing 4, 3, and less than three o-chlorines as described by Jensen and Sundström[9] using Darco G-60 charcoal. The recovery of standard PCBs averaged 95% by this procedure, except in the case of those PCBs having no chlorine in 2,6,2' or 6' positions. The 3,4,3',4' and 3,4,5,3',4',5' polychlorinated biphenyls were only 30–60% recoverable from charcoal columns with benzene eluant. We could also not confirm the ability of undecane to protect PCB against evaporative losses[9]; however, 1,3-propanediol was completely effective in this regard, having the additional advantage that it could be removed from the final solutions by water-washing.

RESULTS AND DISCUSSION

A chromatogram of Aroclor 1260 on Dexsil 410 is shown in Fig. 1. Resolution of complex mixtures is a function both of column efficiency and separation factors. The column efficiency provided by the 50 meter capillary permitted the distinction of components differing in retention time by 3 sec under the conditions used, and permitted the quantitation of peaks separated at their apices by 6 sec. This corresponds to a "closeness criterion" of one retention index unit[11], but when retention indices were calculated by summing half indices we used a closeness criterion of two units. There were a total of 23 peaks that could have been assigned to more than one PCB if retention index on Dexsil 410 was the only criterion. Twenty one of these pairs of PCBs were easily distinguishable on Silar 5C, differing by over ten index units on this polar phase. The other two pairs were distinguishable on Apiezon L. In most cases, the Dexsil-unresolved pairs were isomers having the same number of chlorine atoms, so mass spectrometry would not have enabled them to be separately measured. The use of a second liquid phase differing from the first in selectivity was essential. However, it was not necessary for the second phase to be as efficient as the first. Once
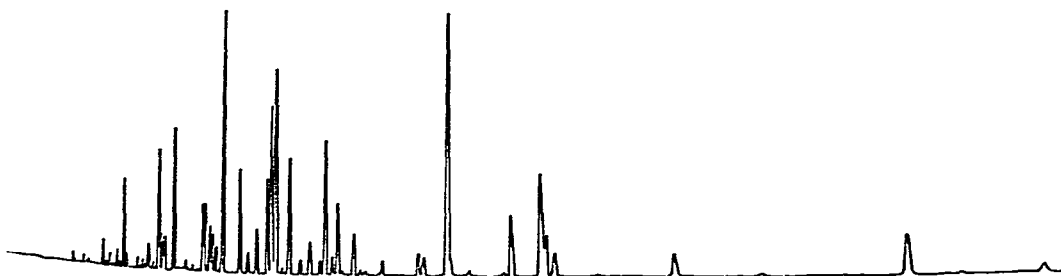

Fig. 1. Separation of Aroclor 1260 on a 50-m glass capillary coated with Dexsil 410. The entire run required 5 h at 200°C with a carrier gas (helium) flow-rate of 0.67 ml/min. The run shown, monitored with an ECD, is attenuated to keep the major peaks on scale. Approximately twenty additional components can be seen at higher sensitivity.

the separation problem has been narrowed down to a distinction between two particular PCBs, one can generally select a liquid phase that can easily make the required separation, if tables of retention indices of PCBs on a large variety of liquid phases are available[11]. Had a packed column or a non-polar capillary column been used as the "master column", however, there would have been groups of more than two components indistinguishable at a closeness criterion of two retention index units.

The compositions of the three Aroclors are summarized in Table I. We have examined several other lots of Aroclor 1254 as well as Kaneclors and Clophens, and it is quite obvious that there are significant quantitative lot-to-lot and brand-to-brand variations. However, there is considerable qualitative similarity. Different lots of Aroclor 1254 resemble each other quantitatively much more than they resemble Clophen A50. For these reasons, the exact numbers in Table I may have some utility in revealing which PCBs are major and which are minor constituents, but they certainly can not be considered accurate representations of all lots of these Aroclors.

The highly toxic PCB, 3,4,5,3',4',5'-hexachlorobiphenyl[1], was sought diligently but not detected even if the columns were grossly overloaded. The less toxic 3,4,3',4'-tetrachlorobiphenyl is apparently present in all the Aroclors except Aroclor 1016 (ref. 12). The pentachloro analogue 3,4,5,3',4', is nearly as strong an inducer of cyto-chrome P-448 as the 3,4,5,3',4',5'-hexachlorobiphenyl[14], but its toxicity has not been evaluated directly. It elutes from non-polar liquid phases such as SF-96 (ref. 9) and Apiezon L[8,9] at positions corresponding to peaks previously unidentified.

Table II gives a comparison of the ECD and HFID responses to those PCBs from Aroclor 1260 amounting to more than 1 % of the total PCB peak areas according to at least one of the detectors. The uncorrected HFID areas are reasonable approximations of the molar percentages for each component; however, it is quite clear from the table that the ECD can not be used to estimate amounts of any individual component in the absence of a calibration standard of that component. Since the PCB mixtures isolated in trace amounts from environmental or biological samples would probably require the halogen-selectivity and sensitivity of the ECD, it would be highly desirable to know the ECD response relative to the HFID response for all PCBs. However, there are a variety of ECDs in common use (^{3}H, ^{63}Ni, pulsed, non-pulsed) and the effects of different carrier/make-up gases and operating parameters on the relative ECD/HFID responses are unknown. Therefore, such relative response factors should probably be determined in each laboratory under the particular conditions that will be applied to environmental samples. This approach to ECD quantitations of PCBs has been employed by Dexter and Pavlou[15], using packed columns.

PCBs do not chromatograph well on liquid phases of extremely high polarity (OV-275, FFAP, etc.) due to their poor solubility in these phases, which results in tailing associated with, presumably, surface adsorption. On the other hand, non-polar columns such as OV-101, in spite of their very high column efficiencies (sharp peaks), do not provide high enough separation factors for optimum fractionation. Thus all PCBs elute from OV-101 between retention indices 1509 and 2760 at 200°C, a span of 1251 units. In contrast, the most polar liquid phase thus far found effective for PCB analysis, polyMPE[11], spreads these components over 1474 index units. Selectivity toward the more highly chlorinated substitution patters may be chosen as needed when phases of moderate polarity are available; for example, the half indices assigned to the 2,3,5,6- and 2,3,4,6-patterns are 1203 and 1200, respectively, on OV-101. This

TABLE I

COMPOSITIONS OF AROCLORS 1248, 1254 AND 1260

Retention indices are determined on Dexsil 410 at 200°C. *o*-Chlorines are from charcoal fractions; no entry = 2 or fewer. Trace means, is present but less than 0.01 %.

Peak No.	Retention Index	o-Chlorines	Chlorine substitution pattern	Molar percentage		
				1248	1254	1260
1	1770		2,2′	0.25	—	—
2	1810		2,5	Trace	—	—
3	1850		2,3′	0.69	0.07	—
4	1863		2,4′	0.18	—	—
5	1922		2,5,2′	9.95	0.07	—
6	1935		2,4,2′	0.19	—	—
7	1958		2,3,2′	0.84	—	—
8	1965		2,6,4′	1.46	—	—
9	1999		2,5,3′	0.75	—	—
10	2018		2,5,4′	9.31	0.72	—
11	2020		2,4,4′	Trace	—	—
12	2023	3	2,5,2′,6′	6.30	0.13	—
13	2046	3	2,3,6,2′	5.73	0.15	—
14	2063		2,3,4′	1.24	Trace	Trace
15	2094		2,5,2′,5′	8.36	4.36	1.91
16	2101		2,5,2′,4′	3.81	1.63	0.44
17	2105		2,4,2′,4′	3.18	0.52	0.08
18	2138		2,4,2′,3′	7.05	2.18	0.66
19	2141	3	2,4,6,2′,5′	1.89	0.29	0.10
20	2155		2,5,3′,5′	2.10	1.01	0.28
21	2160		3,4,4′	1.28	0.20	0.09
22	2168		2,6,3′,4′	0.65	—	—
23	2170		2,3,2′,3′	1.12	0.26	0.04
24	2194		2,4,5,4′	0.25	0.30	0.09
25	2198	3	2,4,5,2′,6′	Trace	Trace	Trace
26	2198	3	2,3,6,2′,4′	1.78	5.00	3.22
27	2208		2,4,6,3′,5′	4.32	3.51	0.57
28	2219		2,5,3′,4′	6.38	4.75	0.85
29	2227		3,5,3′,5′	Trace	Trace	Trace
30	2228		2,4,3′,4′	4.95	2.24	0.22
31	2238		2,3,4,3′	0.11	0.43	0.12
32	2239		2,3,5,2′,5′	0.20	0.63	0.21
33	2243	3	2,3,6,2′,3′	0.71	1.72	0.69
34	2247		3,4,5,2′	Trace	0.18	0.01
35	2253		2,4,5,2′,5′	1.50	6.98	5.04
36	2261		2,4,5,2′,4′	2.52	6.10	0.82
37	2263		2,3,6,3′,5′	3.10	Trace	0.01
38	2276		2,3,3′,4′	Trace	0.18	0.03
39	2284		2,3,5,2′,3′	Trace	0.32	0.09
40	2288	4	2,3,6,2′,3′,6′	0.20	0.34	1.12
41	2294	3	2,3,5,2′,4′,6′	Trace	Trace	Trace
42	2296		3,4,3′,5′	Trace	0.23	0.04
43	2299		2,4,5,2′,3′	0.78	2.59	0.63
44	2303	3	2,3,5,6,2′,5′	Trace	0.33	0.06
45	2308		2,3,4,2′,5′	1.05	3.81	1.10
46	2318	3	2,4,5,2′,4′,6′	—	—	0.14
47	2320		2,3,4,2′,4′	0.55	2.15	0.31

TABLE I (*continued*)

Peak No.	Retention index	o-Chlorines	Chlorine substitution pattern	Molar percentage		
				1248	1254	1260
48	2328		2,4,5,3′,5′	Trace	0.15	3.01
49	2330		2,3,6,3′,4′	1.69	8.51	3.57
50	2338	3	2,3,5,6,2′,3′	0.11	0.38	1.01
51	2339	3	2,3,5,2′,3′,6′	—	0.20	0.29
52	2351	3	2,3,4,6,2′,3′	—	0.14	0.01
53	2355	3	2,4,5,2′,3′,6′	0.77	3.59	9.52
54	2361	4	2,3,5,6,2′,4′,6′	0.12	0.07	0.06
55	2365		3,4,3′,4′	0.47	0.12	0.04
56	2375		Unknown	—	0.31	0.23
57	2379		2,3,4,5,3′	—	0.40	0.06
58	2387		2,3,4,3′,5′	0.02	0.55	0.14
59	2390		2,3,4,5,4′	—	0.25	0.03
60	2395		2,3,5,2′,3′,5′	1.13	0.03	0.06
61	2401		2,4,5,3′,4′	—	8.09	2.00
62	2405		2,3,5,6,2′,3′,6′	—	0.56	0.83
63	2410		2,4,5,2′,3′,5′	—	0.75	1.48
64	2416		2,3,4,2′,3′,6′	Trace	2.00	2.77
65	2419		2,3,4,6,2′,3′,6′	0.09	Trace	0.57
66	2428		2,4,5,2′,4′,5′	0.13	3.32	8.22
67	2436		2,3,4,5,6,2′,6′	Trace	Trace	0.37
68	2444		3,4,5,2′,3′	Trace	0.76	1.88
69	2448		2,3,4,3′,4′	Trace	Trace	Trace
70	2457		2,4,6,3′,4′,5′	0.56	4.23	0.59
71	2466	3	2,3,5,6,2′,4′,5′	—	0.48	1.12
72	2479	3	2,3,4,6,2′,4′,5′	—	1.16	2.58
73	2482		2,3,4,2′,4′,5′	0.19	4.17	5.01
74	2483		2,3,5,6,3′,4′	—	—	Trace
75	2486	3	2,3,4,5,6,2′,5′	—	1.11	5.65
76	2493		2,3,4,6,3′,4′	—	0.46	0.18
77	2500	3	2,3,4,5,6,2′,4′	—	0.28	2.72
78	2508	4	2,3,5,6,2′,3′,5′,6′	—	Trace	0.31
79	2518	3	2,3,4,5,2′,4′,6′	—	Trace	0.47
80	2524	3	2,3,5,6,2′,3′,4′	—	—	Trace
81	2526	4	2,3,5,6,2′,3′,4′,6′	—	Trace	0.15
82	2531	3	2,3,4,6,2′,3′,4′	—	0.30	4.31
83	2540		2,3,4,2′,3′,4′	—	1.31	0.47
84	2542		3,4,5,3′,4′	—	0.16	1.59
85	2546	4	2,3,4,5,6,2′,4′,6′	—	Trace	0.13
86	2557		2,3,4,5,6,3′,5′	—	0.20	0.97
87	2562		2,4,5,3′,4′,5′	—	0.21	0.17
88	2564	3	2,3,4,5,2′,3′,6′	—	Trace	0.09
89	2577	4	2,3,4,6,2′,3′,4′,6′	—	—	0.30
90	2601	4	2,3,4,5,6,2′,3′,6′	—	—	0.38
91	2606		2,3,4,5,3′,4′	—	—	0.41
92	2615		2,3,4,5,6,3′,4′	—	—	0.02
93	2618		2,3,4,5,2′,4′,5′	—	0.76	7.20
94	2620		Unknown	—	Trace	0.04
95	2631		2,3,4,3′,4′,5′	—	0.18	0.03
96	2635	3	2,3,4,5,6,2′,3′,5′	—	1.00	0.15

TABLE I (*continued*)

Peak No.	Retention index	o-Chlorines	Chlorine sunstitution pattern	Molar percentage		
				1248	1254	1260
97	2652	3	2,3,4,5,6,2',4',5'	—	—	0.08
98	2657	3	2,3,4,5,2',3',5',6'	—	—	1.64
99	2671		2,3,5,6,3',4',5'	—	—	2.30
100	2676	3	2,3,4,5,2',3',4',6'	—	—	0.79
101	2680		2,3,4,5,2',3',4'	—	0.43	0.62
102	2689	3	2,3,4,5,6,2',3',4'	—	—	Trace
103	2702	4	2,3,4,5,6,2',3',5',6'	—	—	0.18
104	2733	4	2,3,4,5,6,2',3',4',6'	—	—	1.15
105	2742		Unknown	—	—	0.02
106	2778		2,3,4,5,3',4',5'	—	—	0.13
107	2792		2,3,4,5,6,3',4',5'	—	—	0.01
108	2845		2,3,4,5,2',3',4',5'	—	—	2.21
109	2852		Unknown	—	—	0.11
110	2862	3	2,3,4,5,6,2',3',4',5'	—	—	0.51

TABLE II

COMPARISON OF RELATIVE AREA RESPONSE OF ECD AND HFID FOR MAJOR COMPONENTS OF AROCLOR 1260

Substitution pattern	Molar percentage	Percentage of total peak area	
		ECD	HFID
2,3,6,2',4'	3.22	0.28	2.95
2,4,5,2',5'	5.04	1.11	5.22
2,3,6,2',3',6'	1.12	0.15	1.13
2,3,4,2',5'	1.10	0.38	1.11
2,4,5,2',4',6'	3.15	2.01	3.04
2,3,6,3',4'	3.57	0.99	3.61
3,4,5,3',4'	1.59	2.76	1.65
2,3,4,5,6,3',5'	0.97	1.63	0.85
2,3,4,5,2',4',5'	7.20	13.58	7.45
2,4,5,2',3',6'	9.52	2.63	9.73
2,4,5,3',4'	2.00	1.49	2.04
2,3,5,6,2',3',6'	0.83	1.53	0.74
2,3,4,2',3',6'	2.77	0.87	2.88
2,4,5,2',4',5'	8.22	7.80	9.02
2,3,4,6,2',4',5'	2.58	2.84	2.36
2,4,5,2',3',4'	5.01	5.06	5.28
2,3,4,5,2',4',6'	0.47	1.16	0.44
2,3,4,6,2',3',4'	4.31	4.95	3.63
2,3,4,5,2',3',5',6'	1.64	3.74	1.69
2,3,4,5,2',3',4',6'	0.79	2.80	0.84
2,3,4,5,2',3',4'	0.62	1.50	0.65
2,3,4,5,6,2',3',4',6'	1.15	1.79	1.12
2,3,4,5,2',3',4',5'	2.21	4.36	2.56
2,3,4,5,6,2',3',4',5'	0.51	1.11	0.54
Total	69.59	66.52	70.53

reverses to 1383 and 1390, respectively, on OV-210. Maximum ability to distinguish between these patterns is shown by the liquid phase OV-3, where the respective half-indices are 1240 and 1212. Increased selectivity may in some instances be more important than increased column efficiency in enabling a particular analytical fractionation.

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